The Journal of Nutrition
Supplement: Grapes and Health

Grape Juice, Berries, and Walnuts Affect Brain Aging and Behavior[1–3]

James A. Joseph,* Barbara Shukitt-Hale, and Lauren M. Willis

USDA-Agricultural Research Service, Jean Mayer Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111

Abstract

Numerous studies have indicated that individuals consuming a diet containing high amounts of fruits and vegetables exhibit fewer age-related diseases such as Alzheimer's disease. Research from our laboratory has suggested that dietary supplementation with fruit or vegetable extracts high in antioxidants (e.g. blueberries, strawberries, walnuts, and Concord grape juice) can decrease the enhanced vulnerability to oxidative stress that occurs in aging and these reductions are expressed as improvements in behavior. Additional mechanisms involved in the beneficial effects of fruits and vegetables include enhancement of neuronal communication via increases in neuronal signaling and decreases in stress signals induced by oxidative/inflammatory stressors (e.g. nuclear factor κB). Moreover, collaborative findings indicate that blueberry or Concord grape juice supplementation in humans with mild cognitive impairment increased verbal memory performance, thus translating our animal findings to humans. Taken together, these results suggest that a greater intake of high-antioxidant foods such as berries, Concord grapes, and walnuts may increase "health span" and enhance cognitive and motor function in aging. J. Nutr. 139: 1813S–1817S, 2009.

Introduction

There is a plethora of research that suggests that polyphenolic compounds contained in fruits and vegetables that are rich in color may have potent antioxidant and antiinflammatory activities. In recent published and unpublished studies, e.g., Concord grape juice, blueberry (BB),[4] or strawberry extracts significantly attenuated age-related motor and cognitive deficits. In addition to some reductions in oxidative stress (OS) (i.e. reductions in the imbalance between oxidant production and antioxidant activity; see below), it also appears that there were improvements in other age-related variables concerned with neuronal communication (e.g. receptor sensitivity, signal transduction deficits) that might account for the reductions in behavioral deficits. All plants, including fruit- or vegetable-bearing plants, synthesize a vast array of chemical compounds that are not necessarily involved in the plant's metabolism. These secondary compounds instead serve a variety of functions that enhance the plant's survivability. These compounds may be responsible for the multitude of beneficial effects of fruits and vegetables on health-related issues, 2 of the most important of which may be their antioxidant and antiinflammatory properties. It has been well established that complex mixtures of phytochemicals in fruits and vegetables can provide protective health benefits mainly through a combination of additive and/or synergistic effects and that the matrix factor plays an important role in controlling bioactivity. This is important, because both animals and humans have greater motor and cognitive declines with aging that are related to decreased antioxidant/antiinflammatory protection. Thus, the purpose of this review was to describe the oxidative/inflammatory changes in aging and to show how Concord grape juice, as well as berry fruits and walnuts, may alter some of these changes in aging.

OS

OS results from the shift toward reactive oxygen species (ROS) production in the equilibrium between ROS generation and the antioxidant defense system (1). In the brain, this is particularly important, because there is a long history of studies that have found indications of increased OS in brain aging such as

[1] Published in a supplement to *The Journal of Nutrition*. Presented at the conference "Grape Health Workshop," held in San Francisco, CA, December 2–3, 2008. The supplement coordinator for this supplement is John M. Pezzuto, University of Hawaii at Hilo. Publication costs for this supplement were defrayed in part by the payment of page charges. This publication must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact. The conference was organized by the National Grape and Wine Initiative (NGWI) (its contents are solely the responsibility of the authors and do not necessarily represent the official views of NGWI). *Supplement Coordinator disclosure:* John M. Pezzuto serves as Chair of the Grant Review Committee of the California Table Grape Commission. John M. Pezzuto received an honorarium to serve as moderator at the Grapes and Health Workshop. *Supplement Guest Editor disclosure:* Maria-Luz Fernandez has no relationships to disclose. The opinions expressed in this publication are those of the authors and are not attributable to the sponsors or the publisher, Editor, or Editorial Board of *The Journal of Nutrition*.

[2] Supported in part by Welch Foods, Inc., Concord, MA and supported by the USDA (J.A.J. and B.S.H.). L.W. is supported by NASA and the California Walnut Commission.

[3] Author disclosures: J. A. Joseph, B. Shukitt-Hale, and L. M. Willis, no conflicts of interest.

[4] Abbreviations used: BB, blueberry; IL, interleukin; NFκB, nuclear factor κB; OS, oxidative stress; PG, prostaglandin; ROS, reactive oxygen species; TNFα, tumor necrosis factor-α.

* To whom correspondence should be addressed. E-mail: jim.joseph@ars.usda.gov.

First published online July 29, 2009; doi:10.3945/jn.109.108266.

increases in Bcl-2, which is increased during periods of OS as a mechanism of protection (2), and membrane lipid peroxidation (3). Studies have also shown that there are significant increases in cellular hydrogen peroxide (4). Additionally, there is increased lipofuscin accumulation, a marker of OS (5), along with membrane lipid peroxidation (6).

Early studies suggested that the changes listed above may have induced cell loss in old animals. However, later studies indicated that the age-related changes in neuronal function are much more subtle and may involve calcium dysregulation (7–9). It is important to note here that losses in calcium homeostasis induced by OS and other factors in aging can lead to increased vulnerability to OS and induce a viscous circle of OS, loss of calcium buffering with increased intracellular calcium, and further generation of OS (10–15). Whereas these age-associated changes appear to be dependent upon the particular neurons involved, they all involve various changes in calcium homeostasis and alterations in calcium regulation, with hippocampal and cortical neurons having the greatest alterations (16,17). The consequences of these increases in OS at several levels may result in reduced calcium homeostasis and increased stress signaling cascades [e.g. nuclear factor κB, (NFκB), p-38 mitogen activated protein kinase (see below)]. Additionally, age-related neurodegenerative diseases, such as Alzheimer's and Parkinson's diseases, as well as Huntington's disease all involve losses in calcium buffering and increased OS (18–27).

OS vulnerability in aging may also be the result of microvasculature changes and increases in oxidized proteins and lipids (28), as well as alterations in membrane microenvironment and structure (29,30) and the vulnerability of neurotransmitter receptors to OS (see below). Additional "vulnerability factors" include critical declines in endogenous antioxidant protection involving alterations in the ratio of oxidized:total glutathione (31) and reduced glutamine synthetase (32). Taken together, these findings indicate that there are increases in OS in aging and that the central nervous system may be particularly vulnerable to these increases [see (29,33) for review]. However, even with these findings, it is difficult to discern the mechanisms involved in the increased vulnerability to the OS in aging. It is clear, however, that there is a circle of increased vulnerability leading to decreases in function, which in turn lead to further increases in vulnerability to OS and that perhaps this circle could be broken through the use of nutritional intervention.

Inflammation

Similar results have been reported for inflammation and aging. It has been shown that activated glial cells increase in the normal aging brain, which exhibits greater immunoreactivity in markers for both microglia and astrocytes (34–36). Additionally, increased glial fibrillary acid protein expression is observed by middle age (34), and in the elderly, this increase even occurs in the absence of a defined stimulus (37). Glial cells mediate the endogenous immune system within the microenvironment in the CNS (38) and their activation is the hallmark of inflammation in the brain (39). Activated microglia produce inflammatory molecules such as cytokines, growth factors, and complement proteins (37,40,41). These proinflammatory mediators in turn activate other cells to produce additional signaling molecules that further activate microglia in a positive feedback loop to perpetuate and amplify the inflammatory signaling cascade (42). Activated microglia produce proinflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumor necrosis factor-α (TNFα) (43,44). Research in both aged mice and humans have found increases in TNFα, IL-6 (45–47), and C-reactive protein (48). All of these changes appear to be accompanied by upregulations in downstream indicators of inflammation (e.g. complement, C1q) in microarray studies (49).

Additionally, studies indicate that the expression of cyclooxygenase 2 appears to be associated with amyloid-β deposition in the hippocampus (50,51) and inflammatory prostaglandins (PG) such as PGE increase in the hippocampus, as well as in other areas during aging (52). Because the PG synthesis pathway appears to be a major source of ROS in the brain (53) and other organ systems, these findings indicate that inflammation may be accompanied by, and even generate, its "evil twin," OS, in producing the deleterious effects of aging. Thus, factors such as cytokines and PG may act as extracellular signals in generating additional ROS that are associated with decrements in neuronal function or glial neuronal interactions (54–58) and ultimately the deficits in behavior that have been observed in aging.

Berry fruit, grape, and walnut supplementation in aging

Concord grapes. As mentioned above, previous findings have suggested that improvements in these age-related declines might be accomplished by increasing the dietary intake of polyphenolics in fruits and nuts, especially those identified as being high in antioxidant and antiinflammatory activities. For example, in a previous study (59), we investigated the beneficial effects of 2 concentrations of Concord grape juice (10 and 50%) compared with an energy-matched placebo for their effectiveness in reversing age-related deficits in behavioral and neuronal function in aged Fischer 344 rats. Results showed that rats that drank the 10% grape juice from age 19 to 21 mo had improvements in oxotremorine enhancement of K^+-evoked release of dopamine from striatal slices as well as cognitive performance on the Morris water maze, whereas the 50% grape juice produced improvements in motor function.

These findings suggested that, in addition to their known beneficial effects on cancer and heart disease, polyphenolics present in foods may be beneficial in reversing the course of neuronal and behavioral aging, possibly through a multiplicity of direct and indirect effects that can affect a variety of neuronal parameters. However, the putative relative contributions of the proanthocyanidin and anthocyanin components of the Concord grape juice were difficult to determine, because the juice that was utilized contained both groups of polyphenols. Therefore, in a subsequent study, we assessed the putative role of proanthocyanidins and anthocyanins in these beneficial effects by comparing 30% concentrations of 3 juices: *1*) Concord grape juice, which contains both proanthocyanidins and anthocyanins; 2) Niagara, which contains proanthocyanidins but no anthocyanins; and *3*) Generic White, which contains little to none of these polyphenolic classes and was composed of mostly Thompson seedless grapes. We also tested a control group with a placebo control juice that was matched in energy and acidity to the other juices. Preliminary results suggested that, overall, the grape juice groups and the placebo juice group did not differ on the motor tests, i.e. rats given Concord, Niagara, or Generic White grape juice did not have improved psychomotor function compared with controls. Cognition, as measured by reference memory in the Morris water maze, was significantly enhanced in the Concord and Niagara grape juice groups compared with placebo, whereas the placebo group had improved working memory compared with the Concord and Generic White grape juice groups.

Concord grape effects on memory in humans. However, even given the variability in the 2 animal studies, a preliminary

investigation conducted by Krikorian et al. (60) of the cognitive benefits of grape juice in aged humans has shown that older adults with memory declines, but not dementia, had significant improvements in several measures of cognitive function when supplemented with Concord grape juice for 12 wk compared with the placebo. The placebo was formulated to look and taste like grape juice and to have the same energy load. The verbal memories studied in this randomized, placebo-controlled, double-blind trial had beneficial effects in item acquisition across learning trials on the California Verbal Learning Test, indicating improvement for subjects in the Concord grape juice group relative to those receiving placebo. Thus, although there was variability between the first and second Concord grape studies in animals, it appears that there was some similarity of results between the first Concord grape study and the subsequent human study and that Concord grape juice may be effective in preserving memory function or reversing behavioral deficits in aging.

Berries and walnuts. Concord grape juice, BB (61), strawberries (62), and, most recently, blackberries (63) also enhanced cognitive and motor behavior in aged rats. We observed these findings when we supplemented the berries long term (from 6 to 15 mo of age; F344 rats) (BB or strawberry extract at 2% of the diet); they retarded age-related decrements in cognitive or neuronal function. Results indicated that the supplemented diets prevented the onset of age-related deficits in several indices (e.g. Morris water maze performance) (61). In a subsequent experiment (64), strawberry or BB extract supplementation reversed age-related deficits in neuronal and behavioral (cognitive, Morris water performance, where the rat had to use spatial skills to locate a hidden platform) function in aged (19 mo) F344 rats. A more recent study has suggested that, in addition to Morris water maze performance, BB supplementation was also effective in reversing cognitive declines in object recognition (65). Research has suggested that BB supplementation improved motor performance on tests of motor function that assessed balance and coordination [e.g. rod walking and the accelerating rotarod (64)] and strawberry supplementation produced similar effects on these tasks (62).

Signaling mechanisms. Mechanistically, the putative antioxidant/antiinflammatory relationship with the effects that we have observed is clearly shown in a study in which young rats (3 mo of age) were given a control diet or one supplemented with either BB or strawberry extracts (2% for 8 wk) and then exposed to ^{56}Fe irradiation (1.5 Gy at 1 GeV) (66). We previously showed that these irradiations produce deficits in cognitive and motor behavior similar to those in aging. The results indicated that either strawberry or BB supplementations provided protection against the deleterious effects of radiation on these behaviors. However, the results also suggested that BB supplementation prevented irradiation-induced deficits in memory tasks that depend on intact striatal functioning, such as reversal learning (i.e. when the platform was moved from one quadrant to another in the Morris water maze) and that strawberry-supplemented rats had fewer deficits on the probe trial measures (when the platform was removed from the maze), suggesting retained place information, which is a hippocampus-dependent behavior (66). It appears the polyphenolic compounds in BB may be working mainly in the striatum, while those in strawberries may primarily affect the hippocampus. Regional polyphenol specificity notwithstanding, it also appears that the beneficial effects of these supplementations may involve more than opportunistic free radical scavenging, because assessments of free radicals using 2,7-dichlorofluorescin diacetate in an early study (64) suggested that this activity was limited in the brains of the rats examined.

Subsequent findings from Williams et al. (67) have suggested that these mechanisms involved alterations in cell signaling. They showed that supplementation with a BB diet (2% wt:wt) for 12 wk improved the performance of aged rats in spatial working memory tasks. The performance on these tasks correlated with the activation of cyclic AMP response element binding protein and increases in levels of brain-derived neurotrophic factor in the hippocampus. These changes were associated with increases in extracellular signal-related kinase 1/2, as well as Akt and other signals associated with the synthesis of new protein. This activation would be necessary for memory formation to occur.

Additionally, it also appears that BB supplementation may inactivate stress signals and increase protective signals, because aged male Fischer 344 rats fed a BB-supplemented diet had reduced age-induced increases in NFκB expression in the frontal cortex, hippocampus, and the striatum compared with those of aged unsupplemented controls (65). Similar decreases in NFκB (65) by BB supplementation were produced in animals (4-mo-old rats) given intrahippocampal injections of kainic acid, which is an excitotoxin that produces neuronal lesions and induces an inflammatory response in the brain (68). Gene expression analysis revealed that BB supplementation normalized NFκB to control levels and reduced the expression of the cytokines IL-1β and TNFα in the hippocampus. BB supplementation also increased the expression of the neuroprotective trophic factor insulin growth factor-1. Therefore, the beneficial effects of the berry fruit may involve 4 factors: increases in the activation of protein pathways involved in cognitive function, free radical scavenging, activation of protective signals, and inhibition of stress signals. Interestingly, we also showed that walnut supplementation in the old rats had similar effects on cognitive function as those seen with berry fruit or Concord grape supplementation (69). Preliminary analyses of the stress signals that may be altered with walnut supplementation suggest that the (n-3) PUFA, α-linolenic acid, was very effective in lowering stress signals such as cytokines and NFκB.

Berry effects in humans. The important question, however, is do these findings in berries translate into beneficial effects in humans with respect to learning and memory. Krikorian et al. (60) have recently shown that similar to results in Concord grape juice (59), BB juice supplementations for 12 wk had beneficial effects in humans. The criteria for the participants were the same as those chosen for the Concord grape study. The results indicated that several aspects of learning and memory were improved in the BB juice-supplemented humans, including: list recall, paired associate learning, and intrusion errors. These studies will be continuing and extended to motor behavioral assessments as well. Thus, these findings, as well as those above, suggest that it may be possible to increase "health span" by reversing the deleterious effects of senescence on cognitive and motor behavior via nutritional modulation. This has important implications for improving health in aging populations in the US and other countries.

Other articles in this supplement include (70–76).

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